IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

YAJUN GUO

Serial No.: 08/872,527

Filed: June 11, 1997

For: CELLULAR VACCINES AND IMMUNOTHERAPEUTICS AND METHODS FOR THEIR PREPARATION

Group Art Unit: 1644

Examiner: T. Cunningham

Declaration of Yajun Guo, M.D., Ph.D., under 37 CFR 1.132

1. I, Yajun Guo, Declarant, am the sole inventor of claims contained in the above-captioned patent application. I have extensive training and experience in immunology and related science as demonstrated by my attached CV in Exhibit A.

2. I am familiar with the subject application, including composition and method claims therein directed to immunogenic vaccines. The vaccines are unique because they comprise isolated autologous target diseased cells that contain enhanced levels of primary and/or costimulatory cell surface molecules, and which also have attached bridge molecules that are primed to bind T cells upon administration to a patient. The effect of the vaccine is to physically and functionally unite T cells and tumor cells *in vivo* through the costimulatory and bridge molecules. This double interaction triggers a strong and unprecedented proliferative immune response against tumor cells that occurs when naïve, endogenous T cells are drawn toward, and sensitized by, the vaccine cells, thereby stimulating a mass proliferation of cytotoxic T cells that are proficient at clearing pre-established tumors.

3. I am aware of the pending rejection in the application that is based on a combination of references, namely Wang, Vanky, Renner, Bohlen, Darlington, Chapoval, and Krummel. I have reviewed those references in detail and believe that they are distinguishable from my invention for the following critical reasons.

4. First, the references all fail to recognize the claimed feature and advantage that vaccine tumor cells must be pre-armed with bridge molecules. If they are not, or if soluble bridge molecules are administered separately or sequentially to a patient, or otherwise present in an appreciable amount, the result is a comparatively weak immunogenic response. This is because of the nature of the bridge molecules themselves. These molecules have at least two distinct binding sites, one that is capable of binding tumor cell surface antigens, and another that is specific for T cells. To demonstrate, assume that each T cell and tumor cell has only one site for attachment. Further assume a system that consists of only one tumor cell and one T cell, to which are added two or more bridge molecules. If the two cells upon administration each bind a separate bridge molecule, it is clear that the cells will not then be able to bind *each other* because their respective complementary sites are blocked (mutual site exclusion). In other words, two different cell:BiMab complexes are formed that preclude the two complexes from effectively binding *each other*. This is avoided using the claimed vaccines, which supply only one type of cell:BiMab complex..

5. A second advantage of the invention over the cited art is that vaccine cells according to the claims are isolated, i.e., substantially devoid of responsive T cells to avoid prematurely initiating proliferation and cytotoxicity in vitro. If the vaccine were to contain functional T cells, as the combined prior art teaches, the tumor cells could be destroyed in vitro before ever being able to contribute meaningfully, or optimally, as a vaccine *in vivo*. The claimed invention recognizes and exploits this weakness of the prior art.

6. Yet another advantage of the invention is that the tumor cell surface molecule to which the bridge molecule binds need not be specific for tumor cells, but may be one that is common to many or all cell types in a patient, tumor cells and T cells included. In this way, isolated tumor cells can be "armed" with bridge molecules despite the fact that they are affixed via a nonunique determinant. This has the advantage of greater flexibility and ease in creation of the vaccine. Once affixed, the bridge molecule then endows the armed tumor cell with a specific, artificial affinity for T cells that is mediated by a distinct site on the bridge molecule. This also further illustrates the point that arming should be performed outside the presence of T cells, and once completed, the armed cells further subjected to a purification step that eliminates or substantially reduces unreacted (free, soluble) bridge molecules.

7. The cited art references do not teach or recognize these critical distinctions and consequently fail to provide results that are anywhere near commensurate with results achieved using my invention. I have personally verified this using both in vitro and in vivo animal models. In vitro, I have tested hepa 1-6, SMCC-1, and EL-4 tumor cell lines that, unlike the lines used by Wang and Vanky, e.g., are known to be weak or nonimmunogenic. When I tried to repeat Wang and Vanky's described procedures using these cell lines, I could not induce an immunogenic response. However, when I added the features of my invention, I was very successful.

8. For the in vivo studies, I immunized either with a mixture of cytokine treated tumor and lymphcyte cells with CD28 BiMabs, or with injection of tumor cells followed by intravenous or intraperitoneal BiMab administration. In neither case was an appreciable anti-tumor immune response induced. However, when pre-stimulated, pre-armed, and purified tumor cells were used as a vaccine according to the claimed invention, a strong immunogenic response was observed. Hence my invention, unlike the prior art, is successful in generating strong immunogenic responses against tumor cells that otherwise would be non or weakly immunogenic.

9. Attached as Exhibit B is a series of six figures that demonstrates the significant advantages of my invention over the prior art. As used therein, the term "BsAb" is synonymous with BiMab. Figure 1 shows the general inventive scheme. Figure 2 shows the desirability of not including functional T cells in the vaccine. Figure 3 shows the desirability of minimizing unbound BiMab in the vaccine. Figure 4 shows a more detailed version of the successful inventive scheme that includes in vivo injection. Figure 5 shows the relative ineffectiveness of free BiMab administration and why. Figure 6 demonstrates the enhanced immunogenic and tumoricidal ability of the claimed vaccine in vivo relative to the prior art. As demonstrated, the difference is several fold better (bridged complex + T cells).

10. I declare that all statements made in this Declaration of my own knowledge are true and that all other statements contained herein are made on good information and beliefs that I believe to be true and have no reason to doubt. Further, this Declaration is made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed on September 30, 1999

By: _____
Yajun Guo